(b) washing the IGF-I-loaded first cation exchange matrix with a first cation exchange wash buffer, which removes a substantial amount of adsorbed non-IGF-I material without removing a substantial amount of authentic or non-authentic IGF-I;

(c) eluting all forms of adsorbed IGF-I from the cation exchange matrix of step (a) by contacting said cation exchange matrix with a sufficient quantity of a first cation exchange elution buffer, which has a sufficiently high pH or ionic strength to displace substantially all of said authentic and non-authentic IGF-I from said cation exchange matrix;

(d) transferring the IGF-I-containing eluate from step (c) into an unfolding/refolding buffer, which:

(i) reduces the intrachain disulfide bonds of IGF-I protein and promotes unfolding without permanent denaturation; and

(ii) permits refolding of the IGF-I and reoxidation to form properly-paired intrachain disulfide bonds;

a' cont.

(e) contacting the properly folded IGF-I from step (d), after transfer into a suitable solvent system, with a sufficient quantity of a hydrophobic interaction chromatography matrix under conditions allowing adsorption of at least about 95% of said IGF-I from said eluate;

(f) washing the IGF-I-loaded hydrophobic interaction chromatography matrix with a hydrophobic interaction wash buffer having an ionic strength sufficiently low to remove most of the non-authentic IGF-I, but not so low as to remove a significant proportion of the authentic IGF-I from the hydrophobic interaction chromatography matrix;

(g) eluting the adsorbed IGF-I from said hydrophobic interaction chromatography matrix by contacting said matrix with a hydrophobic interaction elution buffer, which has a sufficiently elevated pH, or sufficiently low ionic strength, to cause displacement of substantially all of the adsorbed authentic IGF-I from said matrix;

(h) contacting the eluate from step (g) with a sufficient quantity of a second cation exchange matrix under conditions allowing adsorption of at least about 95% of the IGF-I from the eluate;

(i) washing the IGF-I-loaded second cation exchange matrix with a cation exchange wash buffer having a sufficiently high ionic strength, or sufficiently high pH, to remove a significant proportion of non-authentic IGF-I, but not so high as to remove a significant proportion of authentic IGF-I;

(j) eluting the adsorbed IGF-I from said second cation exchange matrix by contacting said matrix with a second cation exchange elution buffer, which has a sufficiently high ionic strength, or sufficiently high pH, to displace substantially all of the adsorbed authentic IGF-I from said matrix;

(k) contacting the eluate from step (j), in an aqueous buffer, with a suitable quantity of a reverse phase chromatography matrix under conditions allowing adsorption of at least about 95% of the IGF-I from the eluate;

a1 cont.

(l) washing the IGF-I-loaded reverse phase chromatography matrix with an aqueous/organic reverse phase wash buffer having an organic solvent concentration sufficiently high to remove a substantial proportion of non-authentic IGF-I, but not so high as to remove a significant proportion of authentic IGF-I; and

(m) eluting the adsorbed IGF-I from said reverse phase chromatography matrix with an aqueous/organic buffer having an organic solvent concentration high enough to remove substantially all of the authentic IGF-I without removing a significant proportion of multimeric forms of IGF-I.

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22. (New) A method for refolding an insulin-like growth factor-I (IGF-I) polypeptide derived from a yeast cell medium to yield an authentic, properly folded IGF-I polypeptide comprising denaturing and renaturing IGF-I species present in an IGF-I mixture from said yeast cell medium using a denaturation buffer comprising urea, dithiothreitol, alcohol and salt, in sufficient amounts and under conditions that allow for the reduction and subsequent oxidation of disulfide bonds, thereby producing an authentic, properly folded IGF-I polypeptide.

a2

23. (New) The method of claim 22 wherein the denaturing and renaturing are performed together using a denaturation buffer comprising about 1.5 to about 3 M urea, about 1 mM to about 15